CLAIMS

1. A method of suppressing growth of tumor cells, comprising the step of:

administering to said cells a 14-3-3σ protein having a sequence as shown in SEQ ID NO: 2.

2. A method of suppressing growth of tumor cells, comprising the step of:

administering to said cells a DNA molecule which causes said cells to express 14-3-3σ, said DNA molecule having a sequence as shown in SEQ ID NO: 1.

3. A method for screening potential therapeutic agents for the ability to suppress the growth of tumor cells by activating the expression of 14-3-3σ, comprising the steps of:

incubating a potential therapeutic agent with a cell which contains a 14-3-3σ reporter construct, said reporter construct comprising a 14-3-3σ transcription regulatory region covalently linked in a *cis* configuration to a gene encoding an assayable product;

measuring the production of the assayable product, a potential therapeutic agent which increases the production by the cell of the assayable product being an agent which will suppress the growth of tumor cells by activating the expression of 14-3-3σ.

4. The method of claim 3 wherein the 14-3-3σ transcription regulatory region comprises about 1.815 kb upstream from 14-3-3σ transcriptional start site.

5. The method of claim 3 wherein the 14-3-3σ transcription regulatory region comprises the sequence of SEQ ID NO: 5.

6. A method for diagnosing cancer, comprising the steps of:

testing a tissue to determine if the tissue expresses less 14-3-3σ than normal tissue.

7. The method of claim 6 wherein the step of testing utilizes an antibody which is specifically reactive with 14-3-3σ protein.

8. The method of claim 6 wherein the step of testing utilizes a nucleic acid probe which specifically hybridizes to a 14-3-3σ mRNA, said probe having a sequence

selected from SEQ ID NO:1.

9. A method for diagnosing cancer, comprising the steps of:

testing a tissue to determine if DNA in said tissue contains a mutant 14-3-3σ gene.

10. The method of claim 9 wherein DNA of the tissue is compared to DNA of a normal tissue to determine whether the 14-3-3σ gene is mutant.

11. A 14-3-3σ reporter construct, said reporter construct comprising a 14-3-3σ transcription regulatory region covalently linked in a *cis* configuration to a gene encoding an assayable product.

12. The reporter construct of claim 11 wherein the 14-3-3σ transcription regulatory region comprises about 1.815 kb upstream from 14-3-3σ transcriptional start site.

13. The reporter construct of claim 11 wherein the 14-3-3σ transcription regulatory region comprises the sequence of SEQ ID NO: 5

14. An antisense 14-3-3σ construct comprising:

a. a transcriptional promoter;

b. a transcriptional terminator;

c. a DNA segment comprising one or more segments of the 14-3-3σ gene, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of 14-3-3σ RNA produced by human cells.

15. The antisense 14-3-3σ construct of claim 14 wherein said transcriptional promoter is inducible.

16. A 14-3-3σ antisense oligonucleotide comprising: at least ten nucleotides complementary to 14-3-3σ mRNA.

17. The 14-3-3σ antisense oligonucleotide of claim 16 which comprises at least about twenty nucleotides complementary to 14-3-3σ mRNA.

18. The 14-3-3σ antisense oligonucleotide of claim 16 which contains one or

more modified nucleotide analogs.

19. The 14-3-3σ antisense oligonucleotide of claim 16 which is a circular molecule.

20. A method for promoting the proliferation of cells, comprising the step of:

administering a 14-3-3σ antisense oligonucleotide comprising at least ten nucleotides complementary to 14-3-3σ mRNA to said cells to inhibit the expression of 14-3-3σ.

21. A method for promoting the proliferation of cells, comprising the step of:

administering a 14-3-3σ triplex-forming oligonucleotide comprising at least ten nucleotides complementary to 14-3-3σ gene to said cells to inhibit the expression of a 14-3-3σ gene.

22. A method for promoting growth of cells, comprising the step of:

administering to said cells to inhibit the expression of 14-3-3σ, an antisense 14-3-3σ construct comprising:

a. a transcriptional promoter;

b. a transcriptional terminator;

c. a DNA segment comprising one or more segments of the 14-3-3σ gene, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of 14-3-3σ RNA produced by human cells.

23. The method of claim 22 wherein said transcriptional promoter is inducible.

24. A method for assessing susceptibility to cancers, comprising the step of:

testing a tissue selected from the group consisting of blood, chorionic villi, amniotic fluid, and a blastomere of a preimplantation embryo, to determine if DNA in said tissue contains a mutant 14-3-3σ gene.

25. A method for screening test compounds to identify those which are potential anti-tumor agents, comprising the steps of:

determining DNA content of 14-3-3σ gene-defective human cells incubated

in the presence and in the absence of a test compound, wherein a test compound which causes DNA accumulation in the 14-3-3σ gene-defective cells is identified as a potential anti-tumor agent.

26. The method of claim 25 wherein the DNA accumulation is in excess of four times the cells' haploid DNA content.

27. The method of claim 25 wherein the DNA content of the cells is determined by staining the cells with a DNA-binding dye.

28. The method of claim 27 wherein the DNA-binding dye is selected from the group consisting of H33258 and propidium iodide.

29. The method of claim 27 further comprising measuring the incorporation of the DNA-binding dye by flow cytometry.

30. The method of claim 25 wherein the DNA content of the cells is determined by fluorescence in situ hybridization (FISH).

31. The method of claim 25 wherein the σ-defective human cells are colonic cells.

32. The method of claim 25 further comprising the steps of:

determining DNA content of 14-3-3σ gene-normal human cells incubated in the presence and in the absence of the potential anti-tumor agent;

identifying a potential anti-tumor agent which preferentially causes DNA accumulation in the 14-3-3σ gene-defective cells as compared to the 14-3-3σ gene-normal cells.

33. A method of screening for potential anti-tumor agents, comprising the steps of:

determining viability of 14-3-3σ gene-defective human cells incubated in the presence and in the absence of a test compound;

selecting a test compound which causes cell death in the 14-3-3σ gene-defective cells.

34. The method of claim 33 wherein the viability of the cells is determined by applying a dye to the cell, incorporation of the dye by the cell indicating death of the cell.

35. The method of claim 34 wherein the dye is trypan blue.

36. The method of claim 33 wherein the 14-3-3σ gene-defective human cells are colonic cells.

37. A method of screening for potential anti-tumor agents, comprising the steps of:

determining apoptosis of 14-3-3σ gene-defective human cells incubated in the presence and in the absence of a test compound;

selecting a test compound which causes apoptosis in the 14-3-3σ gene-defective cells.

38. The method of claim 37 wherein the apoptosis of the cells is determined by staining the cells with a DNA-binding dye and observing chromosomes of the cells, condensation of the chromosomes indicating apoptosis of the cells.

39. The method of claim 38 wherein the DNA-binding dye is H33258.

40. The method of claim 37 wherein the apoptosis of the cells is determined by subjecting DNA of the cell to gel electrophoresis, wherein observation of a DNA ladder indicates apoptosis of the cells.

41. The method of claim 37 wherein the apoptosis of the cells is determined by detecting abnormal breaks in DNA of the cells, wherein abnormal breaks in the DNA indicate apoptosis of the cells.

42. The method of claim 41 wherein the abnormal breaks in the DNA are detected by terminal DNA transferase assay.

43. The method of claim 41 wherein the abnormal breaks in the DNA are detected by nick translation assay.

44. The method of claim 37 wherein the 14-3-3σ gene-defective human cells are colonic cells.

45. The method of claim 37 further comprising the steps of:

determining viability of 14-3-3σ gene-normal human cells incubated in the presence and in the absence of the selected test compound;

identifying a selected test compound which preferentially causes cell

death in the 14-3-3σ gene-defective cells as compared to the 14-3-3σ gene-normal cells.

46. A homozygous 14-3-3σ gene-defective human cell line.

47. The homozygous 14-3-3σ gene-defective human cell line of claim 47 which comprises colonic cells.

48. A pair of isogenic cell lines wherein a first cell line is a homozygous 14-3-3σ gene-defective human cell line and the second cell line is a homozygous 14-3-3σ gene-normal human cell line.

49. A method of identifying a chromosome, comprising the step of:

contacting one or more chromosomes with a polynucleotide probe which comprises at least 11 contiguous nucleotides of the sequence shown in SEQ ID NO: 1;

detecting chromosomes which specifically bind to the polynucleotide probe, wherein a chromosome which specifically binds to the probe is identified as containing at least a portion of human chromosome 1.

50. The method of claim 49 wherein the chromosome is identified as containing at least a portion of human chromosome 1p.

51. The method of claim 49 wherein the chromosome is identified as containing at least a portion of human chromosome 1p35.

52. An isolated genomic polynucleotide which comprises at least 20 contiguous nucleotides of the transcription regulatory region of 14-3-3σ as shown in SEQ ID NO: 3.

53. The isolated genomic polynucleotide of claim 52 which comprises BDS-1 (SEQ ID NO: 4).

54. The isolated genomic polynucleotide of claim 52 which comprises BDS-2 (SEQ ID NO: 5).

55. The isolated genomic polynucleotide of claim 52 which comprises 1815 bp upstream of 14-3-3σ's transcription start site.

56. A vector which comprises the genomic polynucleotide of claim 52.

57. A vector which comprises the genomic polynucleotide of claim 53.

58. A vector which comprises the genomic polynucleotide of claim 54.

59. A vector which comprises the genomic polynucleotide of claim 55.

60. A host cell which comprises the vector of claim 56.

61. A host cell which comprises the vector of claim 57.

62. A host cell which comprises the vector of claim 58.

63. A host cell which comprises the vector of claim 59.

64. An isolated intron-free polynucleotide which encodes a 14-3-3σ protein as shown in SEQ ID NO: 2.

65. An isolated intron-free polynucleotide of claim 65 which has the sequence as shown in SEQ ID NO: 1.

66. A vector which comprises the intron-free polynucleotide of claim 65.

67. A host cell which comprises the vector of claim 66.

68. An isolated and purified polypeptide having a sequence as shown in SEQ ID NO: 2.

69. A method for detecting p53 activity in a human tissue, comprising:

contacting (a) a reporter constuct comprising a 14-3-3σ transcription regulatory region covalently linked in a *cis* configuration to a gene encoding an assayable protein product, with (b) a cell lysate of a tissue or a body sample of a human, under conditions suitable to transcribe RNA from the reporter construct and to translate the RNA to form protein;

measuring production of the assayable product; wherein a cell lysate or body sample which increases the formation of assayable product identifies the tissue from which it was made as having wild-type p53.

70. A method for detecting p53 activity in a cell, comprising:

transfecting a test cell with a reporter constuct comprising a 14-3-3σ transcription regulatory region covalently linked in a *cis* configuration to a gene encoding an assayable protein product;

measuring production of the assayable product in the test cell;

comparing the production of the assayable product in the test cell to production of the assayable product in a cell which contains no wild-type p53; wherein a test cell which produces more assayable product than the cell which contains no wild-type p53 is identified as having wild-type p53.

71. A method for detecting p53 activity in a cell, comprising:

transfecting a test cell with a reporter constuct comprising a 14-3-3σ transcription regulatory region covalently linked in a *cis* configuration to a gene encoding an assayable protein product;

subjecting the test cell to a DNA damaging agent;

measuring production of the assayable product in the test cell;

comparing the production of the assayable product in the test cell subjected to a DNA damaging agent to production of the assayable product in a test cell which has not been subjected to the DNA damaging agent; wherein a test cell which produces more assayable product when subjected to the DNA damaging agent than the cell which has not been subjected to the DNA damaging agent is identified as having wild-type p53.

72. A method for detecting the presence of wild-type p53 protein in a cell, comprising the steps of:

contacting a DNA fragment comprising BDS-2 (SEQ ID NO:5) with a cell lysate from a tissue or body sample of a human, to bind the DNA fragment to wild-type p53 present in the cell lysate or body sample;

detecting the presence of wild-type p53 protein in the cell by detecting binding of the DNA fragment to wild-type p53.

73. The method of claim 72 wherein the DNA fragment is labelled with a detectable moiety selected from the group consisting of: a radioactive moiety, a colorimetric moiety, or a fluorescent moiety.

74. The method of claim 72 wherein the step of detecting binding of the DNA framgment to wild-type p53 comprises:

immunoprecipitating p53 protein with anti-p53 monoclonal antibodies.

75. A method of detecting the presence of a wild-type p53 protein in a cell, comprising the steps of:

providing a histological section from a human;

incubating the section with a detectably-labeled DNA fragment which comprises BDS-2 (SEQ ID NO: 5) to bind said DNA fragment to wild-type p53 present in the histological sample;

removing unbound DNA fragment from the histological section; and

determining the amount of DNA fragment which is bound to the histological sample.

76. The method of claim 75 wherein the DNA fragment is labelled with a detectable moiety selected from the group consisting of: a radioactive moiety, a colorimetric moiety, or a fluorescent moiety.

77. A method of identifying compounds which specifically bind to p53-specific DNA binding sequences, comprising the steps of:

contacting a DNA fragment which comprises BDS-2 (SEQ ID NO: 5) with a test compound to bind the test compound to the DNA fragment;

determining the amount of test compound which is bound to the DNA fragment.

78. The method of claim 77 wherein soluble DNA fragments are incubated with the test compound and the p53-specific-binding DNA fragment immobilized on a solid support, said soluble DNA fragments not having the ability to specifically bind wild-type p53 protein.

79. A method of identifying compounds which specifically bind to p53-specific DNA binding sequences, comprising the steps of:

contacting a DNA fragment which comprises BDS-2 (SEQ ID NO:5), said fragment immobilized on a solid support with both a test compound and wild-type p53

protein to bind the wild-type p53 protein to the DNA fragment;

determining the amount of wild-type p53 protein which is bound to the DNA fragment, inhibition of binding of wild-type p53 protein by the test compound indicating binding of the test compound to the p53-specific DNA binding sequences.

80. The method of claim 72 wherein the step of contacting is done in the presence of a non-specific competitor of said DNA fragment.

81. The method of claim 75 wherein the step of incubating is done in the presence of a non-specific competitor of said DNA fragment.

82. The method of claim 77 wherein the step of contacting is done in the presence of a non-specific competitor of said DNA fragment.

83. The method of claim 79 wherein the step of contacting is done in the presence of a non-specific competitor of said DNA fragment.